Hsp90 and Hsp70 chaperones: Collaborators in protein remodeling

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Heat shock proteins 90 (Hsp90) and 70 (Hsp70) are two families of highly conserved ATP-dependent molecular chaperones that fold and remodel proteins. Both are important components of the cellular machinery involved in protein homeostasis and participate in nearly every cellular process. Although Hsp90 and Hsp70 each carry out some chaperone activities independently, they collaborate in other cellular remodeling reactions. In eukaryotes, both Hsp90 and Hsp70 function with numerous Hsp90 and Hsp70 co-chaperones. In contrast, bacterial Hsp90 and Hsp70 are less complex; Hsp90 acts independently of co-chaperones, and Hsp70 uses two co-chaperones. In this review, we focus on recent progress toward understanding the basic mechanisms of Hsp90-mediated protein remodeling and the collaboration between Hsp90 and Hsp70, with an emphasis on bacterial chaperones. We describe the structure and conformational dynamics of these chaperones and their interactions with each other and with client proteins. The physiological roles of Hsp90 in *Escherichia coli* and other bacteria are also discussed. We anticipate that the information gained from exploring the mechanism of the bacterial chaperone system will provide the groundwork for understanding the more complex eukaryotic Hsp90 system and its modulation by Hsp90 co-chaperones.

Heat shock protein 90 (Hsp90) is a highly conserved ATP-dependent molecular chaperone involved in protein homeostasis (1). It is an essential protein in eukaryotes that is known to function in the remodeling of hundreds of client proteins and to participate in many cellular functions, such as protein trafficking, signal transduction, and receptor maturation (1, 2). Additionally, some Hsp90 clients are oncogenic proteins, and their stabilization by Hsp90 can lead to cancer progression, making Hsp90 a potential drug target (1, 3, 4). Although Hsp90 is essential in some bacteria (5, 6), it is not essential in others, including *Escherichia coli* (7). It is a highly abundant protein in both eukaryotes and bacteria under normal nonstress conditions, and its level is further increased during stress conditions (7, 8). To perform protein remodeling functions, eukaryotic Hsp90 acts in conjunction with numerous co-chaperones (1, 9). In addition, some remodeling reactions also require the ATP-dependent chaperone, Hsp70, and Hsp70 co-chaperones (1, 2). In contrast, bacteria lack homologs of the eukaryotic Hsp90 co-chaperones, and protein remodeling can be reconstituted with Hsp90, Hsp70, and Hsp70 co-chaperones (10–14). Understanding the basic mechanism of action of prokaryotic Hsp90 and Hsp70 will contribute to insight into the more complex eukaryotic chaperones.

Structure and conformational dynamics of Hsp90

Domain arrangement of Hsp90 protomers

Hsp90 is a highly conserved protein with ~50% sequence similarity between *E. coli* and humans. Hsp90 exists as a homodimer with each protomer consisting of three domains, including an N-terminal domain (NTD),[4] a middle-domain (MD), and a C-terminal domain (CTD) (Fig. 1, *A* and *B*). The NTD binds ATP, and together the NTD and MD participate in ATP hydrolysis (1, 15–17). A region in the MD interacts directly with Hsp70 in *E. coli* (11) and yeast (18) and may also in higher eukaryotes as well. The CTD possesses the dimerization region (1, 15, 19). Residues in the MD and CTD are involved in interactions with the majority of clients; however, residues in the NTD also participate in binding some specific clients (2, 20–28). Although this three-domain organization is conserved in all Hsp90 proteins, two major features differentiate eukaryotic and bacterial Hsp90 (Fig. 1*A*). One feature is a long, flexible, charged linker that connects the NTD and the MD in cytosolic eukaryotic Hsp90 as well as endoplasmic reticulum Hsp90, Grp94 (15, 29, 30), which is absent in bacterial Hsp90 and mitochondrial Hsp90, TRAP1 (15, 31, 32). This charged linker has been proposed to function as a modulator of Hsp90 conformational changes and co-chaperone binding (33–37). The second distinctive feature is a C-terminal extension containing the MEEVD motif that is present in cytosolic eukaryotic Hsp90

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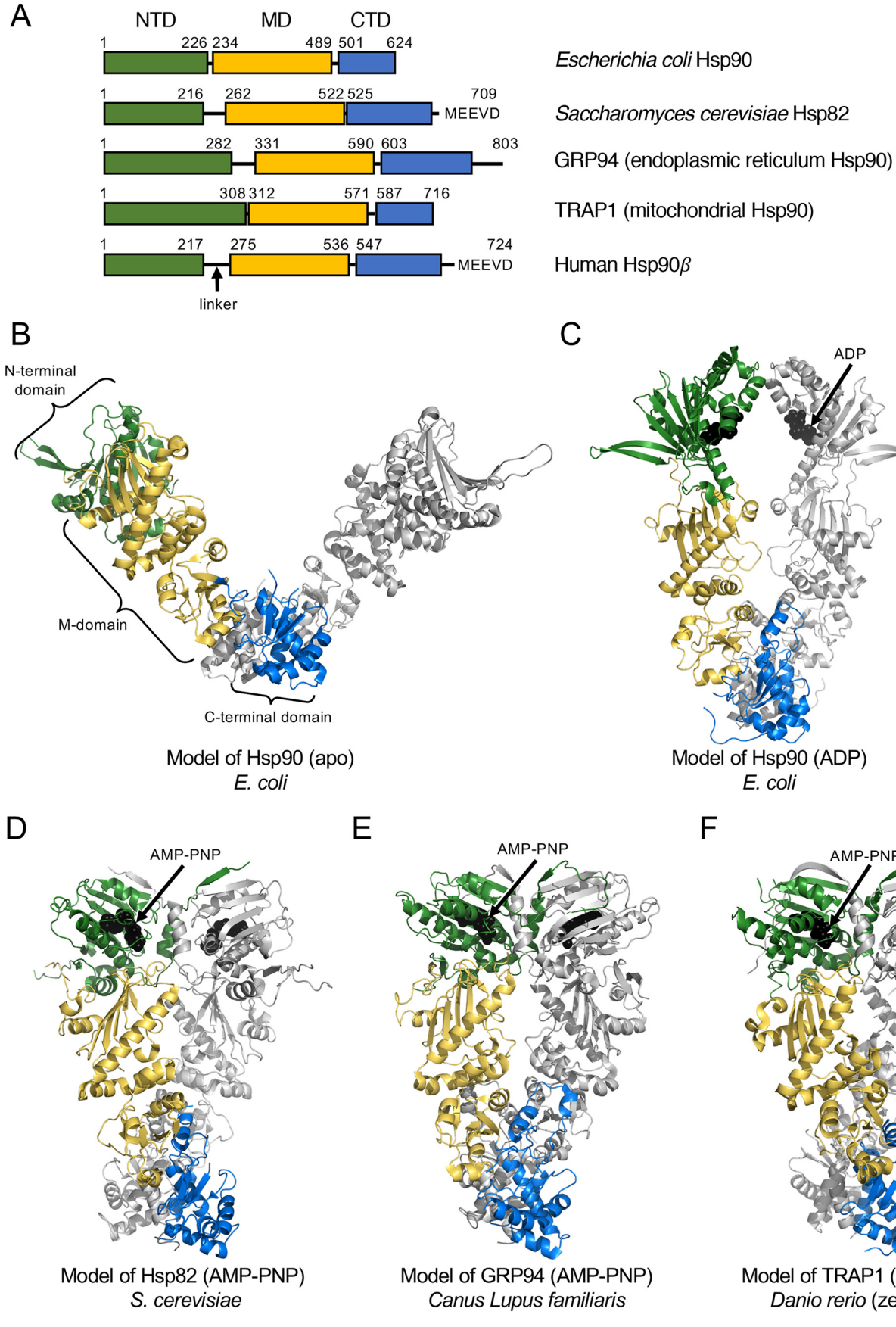
[4] The abbreviations used are: NTD, N-terminal domain; MD, middle-domain; CTD, C-terminal domain; NEF, nucleotide-exchange factor; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; SBD, substrate-binding domain; PDB, Protein Data Bank; NBD, nucleotide-binding domain; TPR, tetratricopeptide repeat; GR-LBD, glucocorticoid receptor-ligand binding domain.


A
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CTD
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282
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590 603
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1
308 312
571 587
716
1
217
275
536 547
724
MEEVD
linker
Escherichia coli Hsp90
Saccharomyces cerevisiae Hsp82
GRP94 (endoplasmic reticulum Hsp90)
TRAP1 (mitochondrial Hsp90)
Human Hsp90β
B
N-terminal domain
M-domain
C-terminal domain
Model of Hsp90 (apo)
E. coli
C
ADP
Model of Hsp90 (ADP)
E. coli
D
AMP-PNP
Model of Hsp82 (AMP-PNP)
S. cerevisiae
E
AMP-PNP
Model of GRP94 (AMP-PNP)
Canus Lupus familiaris
F
AMP-PNP
Model of TRAP1 (AMP-PNP)
Danio rerio (zebrafish)

proteins and allows binding of many co-chaperones containing tetratricopeptide repeat (TPR) domains, including Hop, PP5, CHIP, Tpr2, Sgt1, and Tom70 (Fig. 1*A*) (1, 9, 38). This extension is absent in bacterial Hsp90, Grp94, and TRAP1, consistent with the fact that homologs of the Hsp90 TPR co-chaperones are not found in bacteria and eukaryotic organelles (1, 9, 29, 30).

Dimeric structure of Hsp90

The structure of the Hsp90 homodimer has been solved in multiple nucleotide-bound states as has the structure of Hsp90 in association with various co-chaperones and clients (1, 15, 32, 39, 40). Full-length *E. coli* Hsp90 ($Hsp90_{Ec}$) in the apo, AMP-PNP-, and ADP-bound states revealed three distinctly different conformations (32). Apo-$Hsp90_{Ec}$ predominantly populated an open V-shaped conformation (Fig. 1*B*), and AMP-PNP–bound $Hsp90_{Ec}$ adopted a more closed conformation with the N-domains dimerizing (32). ADP-bound $Hsp90_{Ec}$ populated a twisted compact conformation when studied using negative-stain EM but a less compact form as observed using X-ray crystallography (Fig. 1*C*) (32). The dimeric structures of yeast Hsp90 with AMP-PNP bound (Fig. 1*D*) (41) and GRP94 with AMP-PNP bound (Fig. 1*E*) (30) are similar to that of the $Hsp90_{Ec}$–AMP-PNP dimer (32), suggesting that the conformations may be conserved. Similarly, the apo and ADP-bound conformations are also likely to be conserved (40).

Although the Hsp90 dimer is symmetric in many structures (Fig. 1, *B–E*) (29, 30, 32, 41), a recent structure of mitochondrial Hsp90, TRAP1, in the AMP-PNP–bound state shows an asymmetric conformation at the interface between the MD and CTD (Fig. 1*F*) (31). Because this region is involved in the binding of many clients, the asymmetry may play a role in client interactions (Fig. 1*E*) (20, 26). Conformational asymmetry might be conserved, as small-angle X-ray scattering data has also suggested $Hsp90_{Ec}$ structures exhibit asymmetry (15, 31). Additionally, for eukaryotic Hsp90s, there are many examples of asymmetry within complexes composed of Hsp90 and co-chaperones or clients (1, 2, 15, 21–25, 27, 28, 42–44). Further studies are needed to understand the consequences of these asymmetric complexes on chaperone function.

Conformational dynamics of Hsp90

Taken together, the group of Hsp90 structures clearly shows that Hsp90s are highly dynamic proteins that undergo large conformational rearrangements during the chaperone cycle (Fig. 1, *B–F*) (1, 15, 40, 45). It is important to note that the structures identified for the apo, AMP-PNP-, and ADP-bound states are not the only conformations populated in each condition (39, 40). For example, the apo state consists of a collection of conformations, some more extended (32, 39) and others more compact (39, 40, 46). Moreover, client proteins, and in eukaryotes co-chaperones and post-translational modifications, have also been shown to influence the state that is predominantly populated (15, 42, 45, 47, 48).

The current research supports a model of the Hsp90 ATP cycle that begins with Hsp90 in the open apo conformation (Fig. 1*B*) (1, 15, 32). Upon ATP binding, Hsp90 adopts a closed conformation (Fig. 1, *D–F*) with the N-terminal domains rotating and dimerizing (30–32, 41). In addition, regions of the NTD and the MD within the same protomer interact allowing the ATP hydrolytic site to form (30–32, 41). The recent observation that the ATP-bound conformation, mimicked by AMP-PNP, is asymmetric suggests that ATP hydrolysis may proceed in an asymmetric manner (31, 42, 49). Following ATP hydrolysis and ADP release, Hsp90 again repopulates the open apo form. The asymmetry represents a potential new step in the Hsp90 chaperone cycle, with consequences not only for ATP hydrolysis but also client binding and release and co-chaperone regulation (31, 42, 49, 50).

Hsp90 clients

A large and diverse collection of protein substrates, referred to in the Hsp90 chaperone field as "clients," are remodeled by Hsp90 (1, 2, 15, 17, 51). Although Hsp90 activates numerous kinases, transcription factors, and steroid hormone receptors, many Hsp90 clients are unrelated, both structurally and sequentially. Moreover, results from multiple studies that have identified binding sites for specific substrates of eukaryotic and bacterial Hsp90 point to the overarching conclusion that there are many client-binding sites on Hsp90 (1, 2, 20–28, 42–44, 52). However, many clients, including glucocorticoid receptor-ligand binding domain (GR-LBD) (24, 25), the DNA-binding domain of p53 (21), Cdk4 kinase (28), 50S ribosomal protein L2 (20, 52), and the Δ131Δ fragment of staphylococcal nuclease (20, 26), interact with an extended region formed mainly by residues from the MD and CTD in a cleft between the two protomers of the dimers. In contrast, Tau interacts with a long surface of Hsp90 constituted of residues from the NTD and MD (22). In addition, the affinity of Hsp90 for some clients, including p53 and Tau, is independent of the nucleotide state of Hsp90 (22, 23, 25), whereas other clients, like GR-LBD, bind with higher affinity to the ATP-bound state of Hsp90 (25).

Structures and conformations of Hsp70

Domain arrangement of Hsp70

Hsp70 is a ubiquitous, ATP-dependent molecular chaperone that is involved in a wide array of cellular processes that involve protein folding and remodeling. There is high conservation between human Hsp70 and bacterial Hsp70, referred to as

Figure 1. Domain organization in Hsp90 homologs is conserved. *A,* Hsp90 protomers contain an NTD (*green*) that is involved in ATP binding and hydrolysis and interaction with some clients; an MD (*yellow*) that is involved in client binding and direct interaction with Hsp70; and a CTD (*blue*) that contains the dimerization region and is involved in client binding. A charged, flexible linker of variable length connects the NTD and MD. The C-terminal extension with the MEEVD residues that allow binding with co-chaperones containing a TPR domain is indicated for yeast Hsp82 and human Hsp90*β*. *B,* model of the crystal structure of the *E. coli* Hsp90 dimer in the apo form (PDB code 2IOQ) (32) with the C-terminal domains aligned to the crystal structure of the isolated C-terminal domain (PDB code 1SF8) (19). *C,* dimer structure of *E. coli* Hsp90 in the ADP-bound conformation (PDB code 2IOP) (32). *D,* dimer structure of *Saccharomyces cerevisiae* Hsp82 in the AMP-PNP–bound conformation (PDB code 2CG9) (41). *E,* dimer structure of *Canis lupus familiaris* endoplasmic reticulum Hsp90, GRP94, in the AMP-PNP–bound conformation (PDB code 5ULS) (30). *F,* dimer structure of *Danio rerio* (zebrafish) mitochondrial Hsp90, TRAP1, in the AMP-PNP–bound conformation (PDB code 4IPE) (31). *B–F*, all images were prepared using PyMOL, and the domains are colored as in *A*. *C–F*, bound nucleotide is shown in *black* as CPK models.

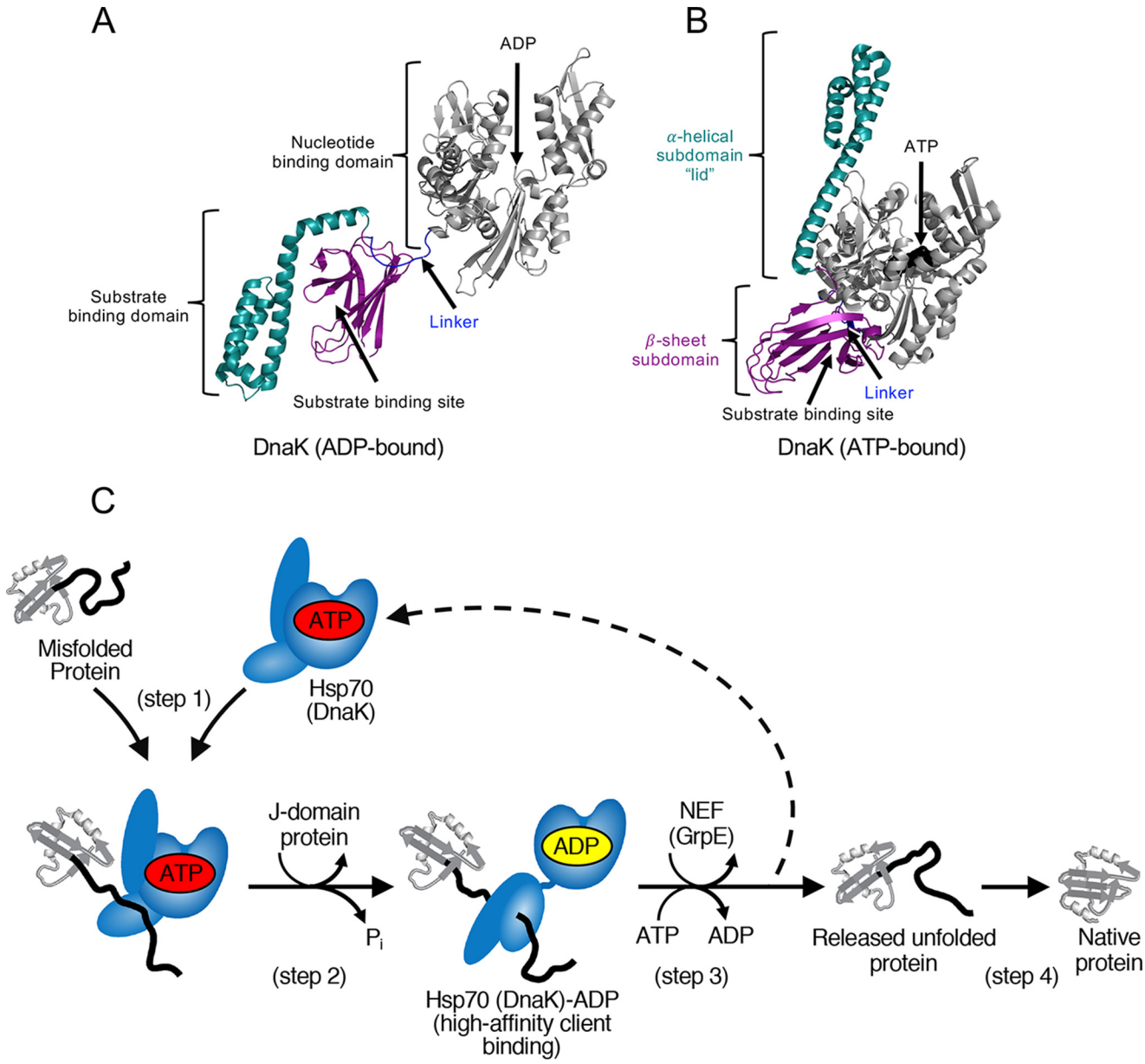

Figure 2. Hsp70/DnaK chaperone. *A* and *B*, structures of *E. coli* Hsp70, DnaK, in the closed (*A*) ADP-bound conformation (PDB code 2KHO) (61) and in the open (*B*) ATP-bound conformation (PDB code 4B9Q), which is stabilized in an oxidized triple mutant (E47C, T199A, and F529C) in the presence of ATP (67). The DnaK protomer contains an NBD (*gray*) connected by a flexible linker (*blue*) to an SBD that is made up of two subdomains, an α-helical subdomain, the "lid" (*teal*), and a β-sheet subdomain that contains the substrate-binding site (*purple*). *B*, ATP is shown in *black* as a CPK model. *C, cartoon* representation of substrate interaction and remodeling by DnaK. First, misfolded substrate interacts with ATP-bound DnaK, which is in the low-affinity substrate– binding state (*step 1*). A J-domain protein, either DnaJ or CbpA, interacts with and stimulates ATP hydrolysis by DnaK (*step 2*), converting DnaK to the ADP-bound high–affinity substrate– binding state. NEF/GrpE promotes nucleotide exchange, from ADP to ATP, via direct interactions with DnaK (*step 3*). The substrate is then released and can fold into the native conformation (*step 4*) or be rebound by DnaK. In *A* and *B* the images were prepared using PyMOL.

DnaK. Hsp70 is composed of an NBD and a C-terminal substrate-binding domain (SBD) with a flexible linker connecting the two domains (Fig. 2, *A* and *B*) (53–56). The NBD is divided into four subdomains, which form a deep cleft where nucleotide binds (Fig. 2, *A* and *B*) (53, 55, 57). The SBD is composed of two subdomains, a β-sheet subdomain that contains the substrate-binding site and an α-helical subdomain that is referred to as the lid (Fig. 2, *A* and *B*) (58–60). In the ADP-bound state with peptide associated, NBD and SBD are tethered together by the linker (Fig. 2*A*) (54, 60–63). In this conformation, the helical lid subdomain of the SBD covers the substrate-binding site (Fig. 2*A*) (58–60, 62). However, solution studies indicate that whereas the helical lid subdomain closes if a peptide is bound to the substrate-binding site, it remains open if the substrate is a large polypeptide, suggesting the importance of lid mobility in accommodating a myriad of substrates (64–66). In the ATP-bound state, the β-sheet subdomain of the SBD is displaced from the α-helical lid, and both subdomains bind to different regions on the NBD leaving the substrate-binding site accessible for rapid, low-affinity substrate interactions (Fig. 2*B*) (67, 68). Additionally, in the ATP-bound conformation the interdomain linker is docked into a crease exposed in the NBD when the α-helical lid of the SBD rotates to interact with the NBD (Fig. 2*B*) (67–69).

Conformational dynamics of Hsp70

Large-scale conformational transitions between the ATP-bound and ADP-bound states of Hsp70 occur during the chaperone cycle (55, 62). However, nucleotide does not lock Hsp70 in one or the other conformation. For example, substrate is able to bind and dissociate from the ADP-bound conformation of Hsp70, suggesting that the substrate-binding site is accessible (55, 62). Similarly, when ATP is bound to Hsp70, the two SBD subdomains may dissociate from their binding sites on the NBD indicating that there are many conformations present (62). Therefore, the open and closed conformational states are stabilized when the specific nucleotide, either ATP or ADP, respectively, is present, but an ensemble of conformations is observable on short time scales under both nucleotide conditions (64, 65, 70, 71).

Hsp70 conformational changes are not only regulated by nucleotide binding and hydrolysis, but also by substrate and co-chaperones (53–55, 62, 67, 72–78). The current model for the *E. coli* Hsp70, DnaK, cycle is shown in Fig. 2*C*. A substrate interacts with DnaK in the low-affinity, open conformation (Fig. 2*C*, *step 1*), leading to a pathway of conformational changes from the SBD to the NBD (55, 56, 66, 79, 80). Substrate-binding and ATP hydrolysis by DnaK are further regulated by two co-chaperones, a J-domain protein and a nucleotide-exchange factor (NEF) (Fig. 2*C*) (53, 72–74, 81–83). The J-domain protein stimulates ATP hydrolysis by DnaK and, in addition, may facilitate the delivery of some substrates to DnaK (Fig. 2*C*, *step 2*) (72, 74, 75, 82–84). There are numerous J-domain homologs in bacteria and eukaryotes, including DnaJ and CbpA in *E. coli* (72, 74). NEF accelerates nucleotide exchange by DnaK, promoting ADP release and ATP binding, which in turn facilitates substrate release (Fig. 2*C*, *step 3*) (53, 72, 73, 81). There is a single NEF in *E. coli*, GrpE, but there are multiple functional homologs in eukaryotes, including Hsp110 and Bag domain proteins (53, 72, 73, 81).

Collaboration between Hsp90 and Hsp70 chaperones

Functional collaboration between Hsp90 and Hsp70 in protein remodeling

The Hsp90 and Hsp70 chaperones isolated from *E. coli*, $Hsp90_{Ec}$ and DnaK, respectively, collaborate in protein reactivation. This relatively simple system has been used to explore the basic underlying mechanism of action of Hsp90 and the interplay between Hsp90 and Hsp70 without the complicating effects of Hsp90 co-chaperones, which are required for protein remodeling by eukaryotic Hsp90. In addition to $Hsp90_{Ec}$ and DnaK, protein reactivation requires the DnaK J-domain co-chaperone, either DnaJ or CbpA (10). The nucleotide-exchange factor GrpE stimulates activity but is not essential (10). Other proteins are not required, consistent with the observations that homologs of the eukaryotic Hsp90 co-chaperones are not present in bacteria. Reactivation requires ATP hydrolysis and client binding by both chaperones, demonstrating that the chaperone functions of both $Hsp90_{Ec}$ and DnaK are required (10).

Using the bacterial chaperones, it has been shown that the DnaK chaperone system acts first without help from $Hsp90_{Ec}$, possibly by binding and stabilizing non-native regions of the substrate, and then DnaK and $Hsp90_{Ec}$ act together to complete client remodeling and reactivation (10, 11). It has been suggested that when the concentration of DnaK is high, DnaK blocks client refolding, and $Hsp90_{Ec}$ acts to relieve that block and promote client folding (13). In both cases, ATP hydrolysis is required during each step (10, 11, 13). Moreover, ATP hydrolysis by the combination of DnaK and $Hsp90_{Ec}$ is synergistically stimulated in the presence of client protein (11, 12, 14). A similar scenario in which Hsp70 acts prior to Hsp90 was also observed using eukaryotic proteins (13, 24, 85–87). However, reactivation by eukaryotic Hsp90 and Hsp70 is stimulated by Hop (Hsp90–Hsp70 organizing protein), an Hsp90 co-chaperone, as well as an Hsp70 J-domain co-chaperone (13, 24, 85–87).

Consistent with Hsp70 acting first on the client protein followed by Hsp90, Hsp70 and Hsp90 recognize clients differently. For example, Hsp70 binds to short, hydrophobic or aromatic sequences that are typically found buried in the hydrophobic core of folded proteins and late folding intermediates but are exposed in unfolded proteins. These sequences occur approximately every 36 residues in most polypeptides (88–90). In contrast, Hsp90 binds to hydrophobic and charged residues exposed over a large surface area, such as natively unfolded proteins, disordered proteins, or late folding intermediates (2, 22, 23, 25, 28, 91).

Direct interaction between Hsp90 and Hsp70

In studies exploring the mechanism of collaboration between bacterial Hsp90 and Hsp70, the two chaperones were discovered to directly interact (10–12, 14). The interaction has been observed both *in vivo* using a bacterial two-hybrid assay and *in vitro* using isolated proteins and several methods, including pulldown assays and BioLayer Interferometry (10–12). Although the interaction is weak, it is stabilized by both client protein and a J-domain co-chaperone (11). Using a genetic screen based on a bacterial two-hybrid assay, $Hsp90_{Ec}$ mutants were identified that were defective in interaction with DnaK. The residues were located in a region of the MD of $Hsp90_{Ec}$ (Fig. 3*A*) (11). Additionally, the mutants were defective in protein remodeling in collaboration with DnaK, showing that the $Hsp90_{Ec}$–DnaK interaction is important for functional collaboration (11). Further work showed that *in vivo* and *in vitro* $Hsp90_{Ec}$ interacts with the DnaK NBD and, more specifically, with a region of DnaK that overlaps with the DnaJ-binding region (Fig. 3, *B* and *C*) (12), which had been previously defined (83, 92–95). The significance of the overlapping sites on DnaK for $Hsp90_{Ec}$ and DnaJ is currently unknown. Additionally, many of the residues in the shared $Hsp90_{Ec}$ and DnaJ site are buried when DnaK is in the ATP-bound conformation, indicating that the ADP-bound form of DnaK interacts with $Hsp90_{Ec}$ (Fig. 3*D*) (55, 67, 68). Consistent with the experimental data, molecular docking showed that the $Hsp90_{Ec}$–DnaK complex involves an interaction between the ADP-bound conformation of DnaK and the apo form of $Hsp90_{Ec}$ (Fig. 3*E*) (12). Notably, in the docked model, client protein bound to the SBD of DnaK is located near the client-binding site of $Hsp90_{Ec}$, making this model functionally realistic (Fig. 3*E*).

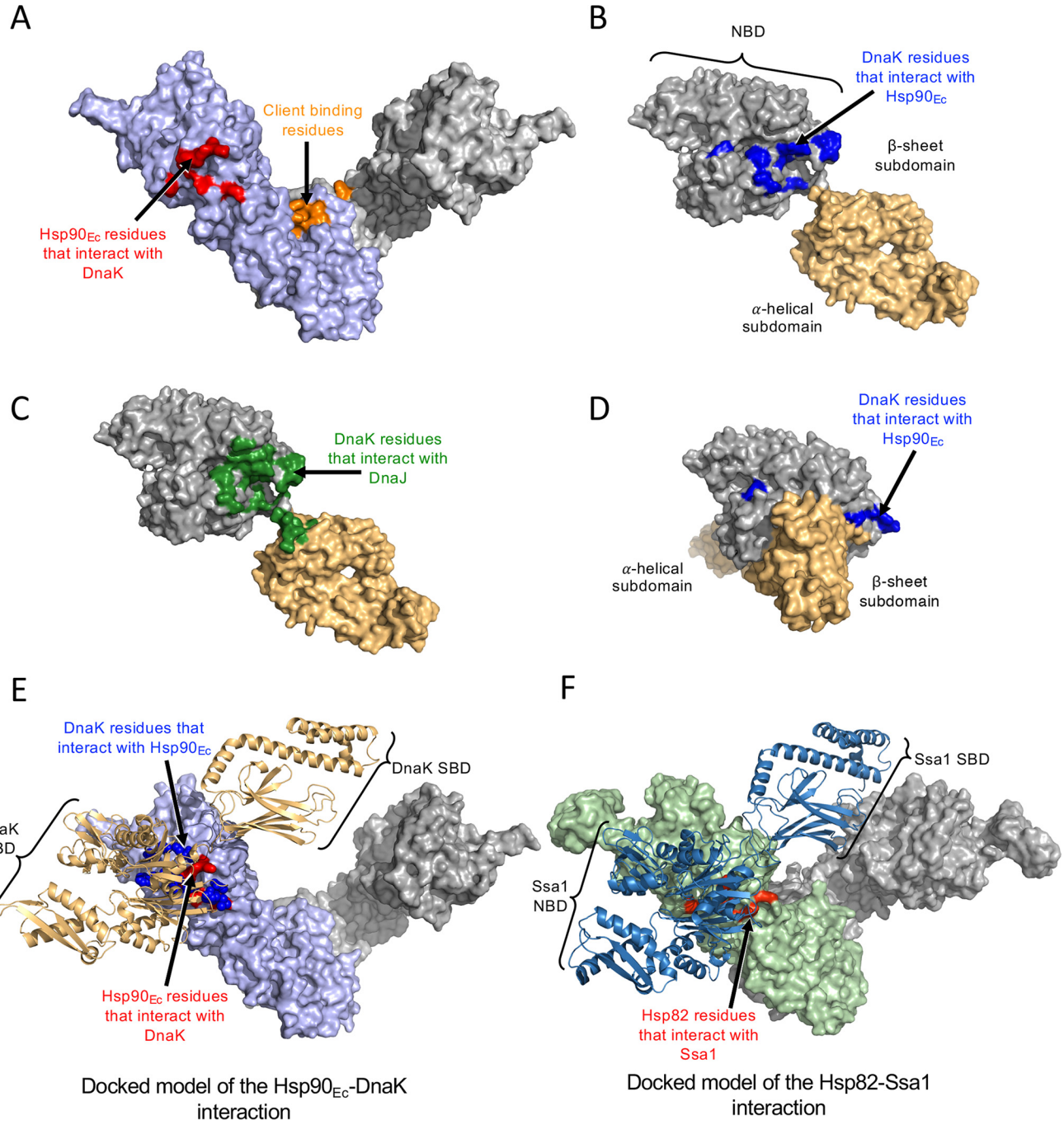

Figure 3. Regions of interaction on Hsp90 and Hsp70. *A,* surface-rendered model of the $Hsp90_{Ec}$ full-length dimer in the apo conformation was built from the biological assembly 1 of PDB code 2IOP (32) using CHARMM to build in missing atoms (12) and shows one protomer in *gray* and the other protomer in *light blue*. The site of DnaK interaction on the MD is shown in *red* (11), and the site of client binding in the MD and CTD is shown in *orange* (20). *B,* surface rendering of DnaK in the ADP-bound conformation (PDB code 2KHO) (61); the NBD is in *gray,* and the SBD is composed of two subdomains, an α-helical subdomain and a β-sheet subdomain in *light orange*. The site of $Hsp90_{Ec}$ interaction on the NBD is shown in *blue* (12). *C,* surface rendering of DnaK in the ADP-bound conformation as in *B* but showing the DnaJ interaction region in *green* (83, 92–95). *D,* surface rendering of DnaK in the ATP-bound conformation (PDB code 4B9Q) (67) showing the change in position of the SBD α-helical and β-sheet subdomains (*light orange*) relative to the NBD (*gray*) and the $Hsp90_{Ec}$-interacting residues (*blue*). *E,* docked surface-rendered model of the apo structure of $Hsp90_{Ec}$ (shown in *A*) and a *cartoon* model of ADP-bound DnaK (shown in *B*) (12). The $Hsp90_{Ec}$ dimer is shown with one protomer in *gray,* one in *light blue,* and the DnaK-interacting region in the MD shown in *red*. DnaK in shown in *light orange* with $Hsp90_{Ec}$-interacting residues in the NBD shown in *blue* as CPK models. *F,* docked homology models of apo yeast Hsp82 and ADP-bound Ssa1 (18). The Hsp82 dimer is shown as a surface-rendered model in *gray* and *light green* with the Ssa1-binding region on the M-domain shown in *red*. A cartoon model of Ssa1 is shown in *blue*. All images were prepared using PyMOL.

Yeast Hsp90, Hsp82, and yeast Hsp70, Ssa1, also directly interact both *in vivo* and *in vitro* (18, 85, 96–99). Moreover, mutational analysis of Hsp82 showed that the site of interaction involves the region of Hsp82 homologous to that of bacterial $Hsp90_{Ec}$, suggesting that the site is conserved in eukaryotes and consistent with a modeled complex of Hsp82 and Ssa1 (Fig. 3*F*) (18). The demonstration that yeast Hsp90 and Hsp70 directly interact was initially unexpected because eukaryotic cells pos-

sess a Hsp70–Hsp90 organizing protein, Hop, in metazoans and Sti1 in yeast (100–102). Hop/Sti1 interacts with both Hsp90 and Hsp70 and was originally thought to act as a bridge between Hsp70 and Hsp90. However, it has been shown that Sti1 stabilizes the weak interaction between Hsp82 and Ssa1 *in vivo* (96, 97) and *in vitro* (18), suggesting that Sti1 facilitates functional collaboration between the two chaperones by stabilizing the interaction. Additional studies show that human mitochondrial Hsp90, TRAP1, interacts with mitochondrial Hsp70, mortalin, in BLI assays *in vitro* (103), consistent with the lack of a Hop homolog in mitochondria. In addition, cytoplasmic human Hsp90β is seen with Hsc70 in pulldown assays (104).

Larger complexes, composed of Hsp90, Hsp70, Hop/Sti1, and client proteins, have been observed using a variety of biophysical techniques, including cryo-EM, EM, or chemical cross-linking followed by MS (24, 28, 100, 105, 106). In these studies, there were differences in the locations of the sites of interaction between the various components and in the conformational arrangements of the proteins, which could indicate that different steps in the pathway of protein remodeling were observed depending upon the technique and proteins used. For example, for GR maturation, differences were observed in the stoichiometry of the Hsp90–Hsp70–Hop–GR complexes with Hsp70 as a monomer (24, 105) or a dimer (106).

Working model for the synergistic action of Hsp90 and Hsp70 in protein remodeling

An illustration of the current working model for the mechanism of protein remodeling by Hsp90 and Hsp70 of bacteria is shown (Fig. 4*A*). It is consistent with the current knowledge of the bacterial chaperones but is still speculative. First, the client protein is bound by DnaK, in a reaction requiring ATP hydrolysis by DnaK and facilitated by DnaJ/CbpA and GrpE (Fig. 4*A*, *step 1*). This is in keeping with previous work showing the DnaK chaperone system functions prior to $Hsp90_{Ec}$ in protein remodeling (10, 11, 13). DnaJ/CbpA has lower affinity for DnaK once ATP is hydrolyzed and likely dissociates from the stabilized ADP-bound DnaK–client complex. Then, DnaK with the bound client directly interacts with a region in the middle domain of $Hsp90_{Ec}$, thus recruiting $Hsp90_{Ec}$ to the ternary complex (Fig. 4*A*, *step 2*). If DnaJ/CbpA remains associated with DnaK following ATP hydrolysis, the overlapping binding region for DnaJ/CbpA and $Hsp90_{Ec}$ on DnaK (12) would likely lead to displacement of the J-protein (Fig. 4*A*, *step 2*). Based on molecular docking results (Fig. 3*E*) (12), a client protein bound to the SBD of DnaK in the ADP-bound conformation is poised for interaction with the client-binding site of $Hsp90_{Ec}$. Binding and hydrolysis of ATP by $Hsp90_{Ec}$ and DnaK trigger conformational changes in the chaperones that lead to client release from DnaK and client binding by $Hsp90_{Ec}$ (Fig. 4*A*, *step 3*). GrpE may aid in nucleotide exchange by DnaK, and DnaK is likely released from $Hsp90_{Ec}$ at this step (Fig. 4*A*, *step 3*). Then, nucleotide dissociation from $Hsp90_{Ec}$ likely causes the release of the client (Fig. 4*A*, *step 4*). The released client may spontaneously refold into the native conformation (Fig. 4*A*, *step 5*) or, in cases where it does not attain its active conformation, it may enter another cycle of remodeling by Hsp90 and Hsp70 or by other chaperones. Models for the mechanism of action of Hsp90 and Hsp70 in eukaryotes have recently been reviewed (1, 2, 15, 17, 23, 27). Although it is likely that the basic mechanism of the collaboration between Hsp70 and Hsp90 is conserved, the eukaryotic systems require the participation of many Hsp90 co-chaperones and thus are significantly more complex than the bacterial system.

Hsp90 holdase activity

In addition to functioning as an ATP-dependent molecular chaperone in collaboration with Hsp70, Hsp90 also functions as a "holdase." Holdase activity refers to the ability of Hsp90 to bind client proteins and prevent their aggregation. This aggregation can be spontaneous, as is seen with α-synuclein, a protein involved in Parkinson's disease pathology (14, 107), or the aggregation can occur during heat treatment of model substrates (14, 108) or following chemical denaturation of model substrates (109). The holdase activity of Hsp90 is independent of ATP binding and hydrolysis (20, 23, 107, 110). Additionally, it does not require Hsp70 in bacteria or eukaryotes and is independent of Hsp90 co-chaperones in eukaryotes (107–109, 111). Some studies have suggested that client release from Hsp90 is independent of ATP (20, 23, 107, 110), although other studies indicate that release of bound clients requires ATP binding and/or hydrolysis (1, 31, 112). The Hsp90–client complexes are transient and reversible, and some clients have been shown to refold into their active conformation following release from Hsp90 (109).

The current model for Hsp90 holdase activity begins with client protein recognition by Hsp90 in a nucleotide-independent manner (Fig. 4*B*, *step 1*). The client is generally an aggregation-prone protein, such as an amyloidogenic protein, a natively unfolded protein, or an unstructured polypeptide formed by chemical or heat treatment (1, 14, 107–109, 111). The Hsp90 dimer binds client with a 1:1 stoichiometry and holds the client, thus preventing protein aggregation or assembly (Fig. 4*B*, *step 1*) (14, 107, 109). Client proteins may be released as Hsp90 transitions through its various conformations, either in an ATP-independent manner or for some clients through ATP-dependent transitions (Fig. 4*B*, *step 2*). Following release, the unstructured regions of the client refold spontaneously into the native conformation (Fig. 4*B*, *step 3*). If the native conformation is not attained, the client may undergo repeated cycles of binding and release by Hsp90 until conditions for refolding are favorable, such as a return to nonstress conditions.

In vivo functions of bacterial Hsp90s

E. coli

Although $Hsp90_{Ec}$ is not essential for growth, strains lacking the gene encoding $Hsp90_{Ec}$, *htpG*, exhibit several phenotypes. For example, growth at very high temperature is slightly decreased in a Δ*htpG* strain compared with a WT strain (7, 113), and a small decrease in viability following heat shock at 50 °C is observed in a Δ*htpG* strain compared with WT (113). In addition, there is a slight increase in the amount of aggregated proteins in a heat-stressed Δ*htpG* strain compared with WT (113). It has also been observed that Δ*htpG* strains have

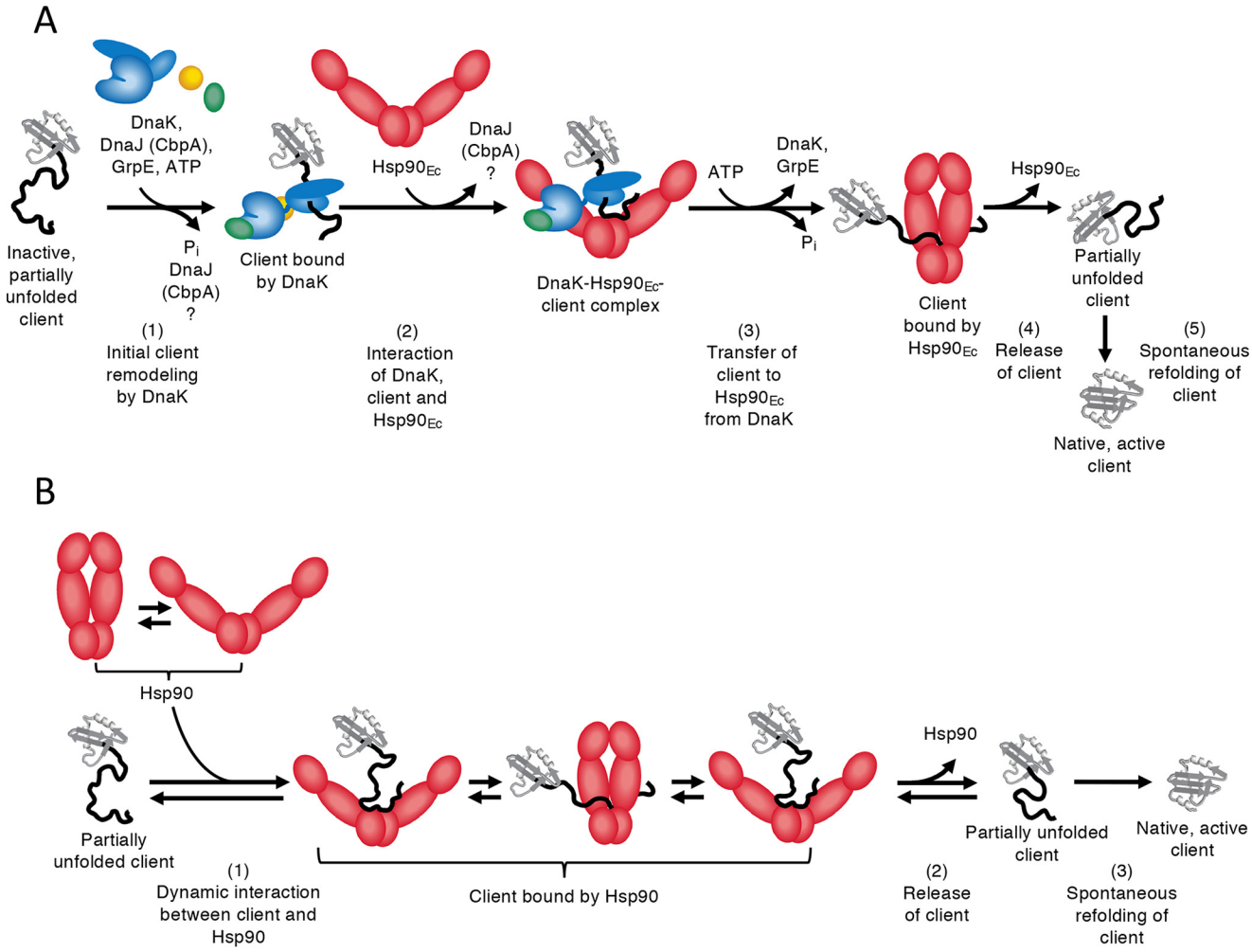

Figure 4. Models for the mechanisms of action of Hsp90. *A,* working model for protein remodeling by *E. coli* Hsp90 in collaboration with the *E. coli* Hsp70 system. First, DnaK binds client protein and in a process that requires a J-protein (DnaJ or CbpA), nucleotide-exchange factor (GrpE), and ATP initially remodels the client (*step 1*). Next, DnaK (in the ADP-bound conformation) recruits $Hsp90_{Ec}$ (in the apo conformation) to the client via a direct interaction between the DnaK NBD and the M-domain of $Hsp90_{Ec}$ (*step 2*). Because DnaJ (CbpA) interaction with DnaK in the ADP-bound conformation is weak, DnaJ (CbpA) is likely released at this step; $Hsp90_{Ec}$ and DnaJ (CbpA) share an overlapping binding site on DnaK suggesting that if DnaJ (CbpA) remains bound to ADP-bound DnaK, $Hsp90_{Ec}$ may displace it. Client is transferred to $Hsp90_{Ec}$ from DnaK following nucleotide exchange by DnaK (to the ATP-bound conformation) and ATP binding by $Hsp90_{Ec}$ (*step 3*). The ATP-bound conformation of DnaK likely dissociates from $Hsp90_{Ec}$ and conformational changes by ATP-bound $Hsp90_{Ec}$ lead to stabilization of client binding. After ATP hydrolysis and ADP release by $Hsp90_{Ec}$, client protein is released (*step 4*) and can reach its active conformation either spontaneously (*step 5*) or it may reenter the chaperone cycle. *B,* working model for the holding activity of Hsp90. First, the aggregation prone client or folding intermediate is recognized by Hsp90 (*step 1*). Client recognition can be through one of many different client-binding sites on Hsp90 and is nucleotide-independent. The client is held by Hsp90 and prevented from aggregating by Hsp90 through a 1:1 stoichiometric interaction. Again, nucleotide is not required for this step. Next, for some clients, release occurs transiently, without the need for nucleotide, but for other clients, ATP binding, hydrolysis, and/or release may be necessary (*step 2*). The client may fold to its native conformation spontaneously (*step 3*), or it may rebind Hsp90.

reduced swarming ability (114), are partially defective in swimming (115), and are slightly defective in biofilm formation at high temperature (116). Additionally, some proteins are less stable in Δ*htpG* strains compared with WT, suggesting a role of Hsp90 in the prevention of degradation. For example, Δ*htpG* strains are defective in CRISPR function because the level of Cas3, an essential component of the CRISPR system, is lower than in the WT strain (117). Similarly, extraintestinal pathogenic *E. coli* lacking *htpG* are nonvirulent and do not produce the genotoxin colibactin and the siderophore yersiniabactin, unless the strain is also lacking the HslUV protease (118). This observation suggests that $Hsp90_{Ec}$ protects a protein required for colibactin and yersiniabactin production from degradation (118). However, the precise role of $Hsp90_{Ec}$ in all of the phenotypes mentioned is still unknown.

Additional bacteria

In bacteria other than *E. coli*, Hsp90 has been shown to be essential under specific stress conditions. For example, in the cyanobacterium *Synechoccocus* sp. Hsp90 is essential at high temperature, although it is dispensable at permissive temperature (6). Additionally, in *Synechoccocus*, Hsp90 is associated with cold tolerance (119) and resistance to oxidative stress (120). Moreover, some clients of *Synechoccocus* Hsp90 have been identified, including HemE, an enzyme important for tetrapyrrole biosynthesis (121, 122) and the rod linker protein of a large protein complex, the phycobilisome, which is essential for photosynthesis (123).

The aquatic γ-proteobacterium *Shewanella oneidensis* also has a requirement for Hsp90 at high temperature, although it is

not needed at permissive temperature (5). In *S. oneidensis*, $Hsp90_{So}$ interacts with an essential protein involved in tRNA maturation, TilS, and at high temperature, TilS activity strictly depends on Hsp90 (5). A potential role of Hsp90 in cold adaptation for γ-proteobacteria, including *Shewanella* sp., was also investigated by looking for Hsp90-interacting proteins (124). Multiple potential client proteins were identified, but the role of these proteins and Hsp90 in cold adaptation requires further investigation.

As observed for certain pathogenic *E. coli* strains, Hsp90 participates in the virulence of other pathogenic bacteria, including *Salmonella typhimurium* (125), *Edwardsiella tarda* (126), *Pseudomonas aeruginosa* (127), and the phytopathogenic bacteria *Xanthomonas albilineans* (128). However, the molecular mechanism for Hsp90 in these processes is still unknown. Additionally, the phenotypic observations made in these studies need further investigation to identify the pathways and client proteins altered in the presence and absence of Hsp90.

Concluding remarks

In the past several years, multiple approaches by many laboratories have led to significant progress toward understanding how Hsp90 and Hsp70 function individually in protein remodeling and how they function collaboratively. Studies using bacterial Hsp90 and Hsp70 have been instrumental in demonstrating some of the essential elements of the interplay between Hsp90 and Hsp70. These studies are also providing a foundation for building a better understanding of the modulation of eukaryotic Hsp90 and Hsp70 by co-chaperones, post-translational modifications, and clients. A better understanding of the collaboration between the Hsp90 and Hsp70 chaperones and their co-chaperones will be important for designing inhibitors that target Hsp90, Hsp70, and interacting proteins for the treatment of diseases that range from cancer to Alzheimer's and Parkinson's disease.

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